Original Article

Evaluation of serum and pleural levels of the tumor markers CEA, CYFRA21-1 and CA 15-3 in patients with pleural effusion*

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Abstract

Objective: To determine the levels of the tumor markers carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA21-1) and carbohydrate antigen 15-3 (CA 15-3) in the blood and pleural fluid of patients with benign or malignant pleural effusion, evaluating the sensitivity of each marker in these fluids. **Methods:** We prospectively evaluated 85 patients with pleural effusion. The study of the pleural fluid observed the criteria established in the literature. Levels of the markers were determined using electrochemiluminescence. The sensitivity was determined on the condition that the specificity was ≥ 90%. **Results:** Of the 85 cases, 36 (42.4%) were malignant, 30 (35.3%) were benign, and the results were inconclusive in 19 (22.3%). In the malignant cases, the CEA and CYFRA21-1 levels were higher in the pleural fluid than in the blood, which was not observed for CA 15-3. In the benign cases, the CYFRA21-1 levels were higher in the pleural fluid than in the blood, whereas the opposite was found for CEA and CA 15-3. There were significant differences between malignant and benign cases for all markers, in pleural fluid and blood. In the pleural fluid, the sensitivity of CEA, CYFRA21-1 and CA 15-3 was 69.4, 69.4 and 66.7%, respectively, and the combined sensitivity was 80.6%. In the blood, the sensitivity was 57.1, 71.4 and 48.6% for CEA, CYFRA21-1 and CA 15-3, respectively, and the combined sensitivity was 77%. **Conclusion:** The results suggest that these markers might be useful in the differentiation between malignant and benign pleural effusion.

Keywords: Biological tumor marker; Cyfra 21.1; CEA; CA 15.3; Pleural effusion.

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Submitted: 5 January 2006. Accepted, after review: 31 July 2006.

Introduction

The etiological diagnosis of pleural effusion is frequently a problem in clinical practice, especially in terms of the differentiation between malignant and benign pleural effusion, due to the significant difference in the treatment and prognosis involved.[1] Statistics for the United States show that the annual number of new cases of pleural effusion is over 1,000,000, approximately 200,000 of which are found to be associated with a malignant disease.[2,3] The majority of neoplasms can cause pleural effusion during their progression. The malignant disease most often associated with pleural effusion is lung cancer, which accounts for up to 30% of all cases of malignant pleural effusion.[4] Lung cancer is followed by, in order of frequency, breast cancer and the lymphomas, which, when accompanied by lung cancer, account for 68% of all cases of malignant pleural effusion.[3] The cytopathologic study of the pleural fluid is the diagnostic method most often used in the identification of malignant pleural effusion and has a sensitivity of approximately 50%, which can be increased by up to 30% if needle biopsy of the pleura is performed.[1,5] Due to the low sensitivity of the method, the results can be inconclusive in terms of the identification of malignancy, and invasive procedures such as thoracoscopy might be necessary.[2,3]

In the attempt to improve the identification of malignant cases, various studies have reported the usefulness of biological tumor markers.[6-9] These tumor markers are macromolecules produced by neoplastic cells or whose production is increased in the presence of neoplasia.[10] These markers can be detected in various biological specimens such as blood, serous liquid, and tissue samples.[8] The markers present different sensitivity and specificity according to the histological type of the primary tumor. Therefore, cytokeratin 19 fragment (CYFRA21-1) presents good sensitivity for non-small cell bronchogenic carcinomas, especially those of the squamous type[5,11]; levels of carbohydrate antigen 15-3 (CA 15-3) are often elevated in patients with advanced breast cancer[11]; and levels of carcinoembryonic antigen (CEA) are elevated in various malignant diseases, especially those of epithelial origin.[8] Studies have demonstrated that combining tumor markers improves their sensitivity in the diagnosis of malignant effusion.

Although there are a number of studies in the international literature involving the analysis of biological tumor markers in pleural fluid as a means of identifying associations with thoracic neoplasms, only a few such studies have been conducted in Brazil,[6,12] and we found no studies in which biological tumor markers were analyzed in more than one type of biological material simultaneously.

Therefore, our objective was to evaluate the determination of the tumor markers CEA, CYFRA21-1 and CA 15-3 in blood and pleural fluid of patients with pleural effusion without previous diagnostic investigation, as well as to assess their usefulness as auxiliary methods of differentiating between benign and malignant cases.

Methods

Population studied

The population was composed of 85 consecutive patients referred to the pulmonology sector of a university hospital, over an eight-month period, for the investigation of pleural effusion. All patients agreed to participate in the study and gave written informed consent. Three patients refused to allow the collection of blood samples but agreed to allow the collection of biological material through pleural puncture. None of the patients had previously been investigated in terms of the pleural effusion, nor were any of them being treated for malignant disease (with chemotherapy or radiotherapy) or for tuberculosis (with anti-tuberculosis drugs). An established questionnaire was used to collect data regarding demographics (age, gender, and profession), smoking (past and present),[13] the aspect of the pleural fluid, respiratory symptoms, information on past/present diseases, complimentary exams, and recent/present use of medication. In the differentiation between exudates and transudates, we used the criteria established by Light.[14] The effusion was considered malignant when the cytopathologic study of the pleural fluid or the anatomopathological study of the pleural samples indicated malignancy. The pleural effusion was considered benign when the cytopathologic study of the pleural fluid or the anatomopathological study of the pleural samples was negative for malignancy, as well as when the accumulation of pleural fluid was accompanied by one the following conditions:

a) Parapneumonic effusion or empyema, according to Light[15];

b) Tuberculosis: patients in whom the biopsy findings were suggestive of active tuberculosis with caseating granuloma and the cytology of the pleural fluid revealed a predominance of lymphocytes or an adenosine deaminase level > 40 IU;
c) Congestive heart failure: the study of the fluid revealed transudate or exudate, in patients with decompensated heart failure; and
d) Liver disease: transudative pleural effusion in patients with liver disease, with no other identifiable cause of the pleural effusion.

The evaluation of the pleural effusion was considered inconclusive when the cytopathologic study of the pleural fluid and the morphologic examination of the pleura did not indicate the cause of the pleural effusion. These cases were not included in the determination of the markers.

Collection of biological material

Blood samples were simultaneously collected to determine the levels of tumor markers and the biochemical levels of lactate dehydrogenase, albumin, and total proteins. Percutaneous thoracic puncture was used to collect the pleural fluid, which was sent for biochemical testing, leukocyte measurement, culture, and smear cytology. Pleural biopsy was performed in the exudates for an anatomopathological study. Serum and pleural fluid were stored in aliquots of 10 mL at −80 °C for later determination of the tumor marker levels.

Determination of tumor marker levels

The electrochemiluminescence method was used to determine the levels of CYFRA21-1, CA 15-3, and CEA. This was achieved with an immunoassay analyzer (Elecsys® 2010; Roche, Mannheim, Germany) and commercial kits for each marker. When the level of a given marker surpassed the detection limit of the method, a dilution of 1:100 was used. The values established for the respective markers in the serum (according to the manufacturer) were as follows: CEA = up to 5 ng/mL; CYFRA21-1 = up to 3 ng/mL; and CA 15-3 = up to 40 ng/mL.

Statistic methodology

In the data analysis, associations between categorical variables were evaluated using the Fisher-Freeman-Halton exact test.[16] The comparison among the values for each marker, in serum and in pleural fluid, was carried out using the (nonparametric) sign test. The comparison between the values for two markers, in serum and in pleural fluid, was carried out using the Mann-Whitney test. To establish the sensitivity of the markers, a cut-off point was determined for each, using a receiver operating characteristic (ROC) curve, under the condition of specificity equal to or greater than 90%. In the construction of the ROC curves, the patients with malignant effusion were considered cases, and the patients with benign effusion were considered controls. The effectiveness of the combination of the three markers to distinguish between malignant and benign effusion was evaluated by constructing multivariate logistic regression models.[17] In the construction of these models, the three markers were considered independent variables. The response variable was composed of the two categories of pleural effusion (malignant or benign), and malignant effusion was the category of interest from the prediction point of view. In all tests, the level of significance that would allow the rejection of the null hypothesis was set at $p < 0.05$.

Ethical aspects

The study design was approved by the Oswaldo Cruz University Hospital Committee for Ethics and Research (protocol nº. 196/96).

Results

Of the 85 patients studied, 36 (42.4%) were diagnosed with malignant pleural effusion, and 30 (35.3%) were diagnosed with benign effusion. The results were inconclusive in 19 (22.3%).

Table 1 shows the distribution of the patients by age and gender. The etiologic diagnoses of the cases of pleural effusion can be seen in Table 2.

The inconclusive cases were not evaluated. Approximately 27% of the malignant cases were associated with lung tumors and 13.8% with breast tumors. In the benign cases, tuberculosis prevailed. The cytology of the pleural fluid provided the diagnosis in 25 (69.4%) of the cases of malignant pleural effusion.

Table 3 shows the medians, together with the first and third quartiles, for the pleural fluid/serum

Table 1 - Distribution of the final diagnosis by age and gender.

	Pleural effusion diagnosis		
	Malignant (n = 36)	Benign (n = 30)	Inconclusive (n = 19)
Variable			
Age (years)	58.9 (14.3)	40.9 (18.6)	56.9 (19.0)
Gender			
Male	18 (37.5%)	18 (37.5%)	12 (25.0%)
Female	18 (48.7%)	12 (32.4%)	7 (18.9%)

Data presented as mean and standard deviation.

tumor marker levels in the malignant and benign cases.

In the patients with malignant pleural effusion, the CYFRA21-1 and CEA levels were significantly higher in the pleural fluid than in the serum (sign test: $p < 0.001$ and $p = 0.014$, respectively). However, there was no statistically significant difference between the serum and pleural fluid in terms of the levels of CA 15-3 (sign test: $p = 1.00$).

In the cases of benign pleural effusion, CYFRA21-1 levels were significantly higher in the pleural fluid than in the serum (sign test: $p < 0.001$). However, the levels of CEA and of CA 15-3 were significantly lower in the pleural fluid.

Table 2 - Distribution of the cases of malignant and benign pleural effusion by etiology.

Causes	n
Malignant	35
Secondary to carcinomas	
Lung	10
Mediastinal	1
Prostate	2
Kidney	1
Breast	5
Other	9
Secondary to other tumor types	
Lymphoma	2
Mesothelioma	3
Melanoma	1
Other	2
Benign	30
Tuberculosis	22
Nonspecific infections	4
Liver disease	2
Heart disease	2

There were statistically significant differences between the malignant and benign cases in terms of the pleural fluid/serum levels of CYFRA21-1, CEA, and CA 15-3 (Mann-Whitney test: $p < 0.001$), the only exception being serum levels of CA 15-3 ($p = 0.045$).

The sensitivity and specificity of each marker are represented in Table 4. The cut-off points for each marker were chosen based on their respective ROC curves, under the condition of specificity equal to or greater than 90%. Under that condition, it was determined that, in the pleural fluid, the greatest sensitivity was achieved with the combination of CYFRA21-1 and CEA (69.4% for both). When all three markers were used in conjunction, the sensitivity increased to 80.6%.

In the blood, CYFRA21-1 presented the greatest sensitivity (71.4%). When the three markers were combined, the sensitivity rose to 77%.

There were no statistically significant differences among the smokers, former smokers and nonsmokers in terms of the levels of any of the markers, in serum or in pleural fluid (Kruskal-Wallis test; $p > 0.462$ for all comparisons).

Discussion

Recognizing patients with pleural malignancy if fundamental, since, unlike those with the benign form, for whom the prognosis is favorable, such patients have a mean survival of three to six months.[18] Thoracentesis with cytopathologic study of the pleural fluid, the principal diagnostic method, presents great variation in its sensitivity (40-60%),[1,2,18] which is increased by up to 7% when a pleural biopsy is also performed.[14] Although surgical procedures (thoracoscopy and thoracotomy) present better diagnostic sensitivity for malignant cases (90%), they are expensive and are not available at all medical centers.[20]

Of the 85 patients evaluated in the present study, 92.9% presented pleural exudate. There was a discrete predominance of malignant pleural effusion (42.4%) over benign pleural effusion (35.3%). These results are similar to those found in the literature, reflecting the frequency of neoplastic pleural effusion, which can account for as much as 50% of the pleural exudates.[21] The distribution found might be related to the fact that this study was carried out at a facility that is a regional referral

Table 3 - Median, first and third quartiles of the markers CYFRA21.1, CEA and CA15-3, obtained in the serum and pleural fluid of the patients with malignant or benign pleural effusion.

Marker	Pleural effusion							
	Malignant				Benign			
	Pleural fluid (n = 36)		Serum (n = 35)		Pleural fluid (n = 30)		Serum (n = 28)	
	Median	Q1-Q3	Median	Q1-Q3	Median	Q1-Q3	Median	Q1-Q3
CYFRA21.1	101.1	25.2-478.3	6.4	2.4-17.3	12.2	6.9-23.2	1.0	0.7-1.9
CEA	27.6	0.8-496.8	4.6	1.0-17.7	0.4	0.2-1.2	0.9	0.3-1.7
CA 15-3	43.9	19.4-167.4	28.6	13.6-81.3	15.9	9.0-20.7	19.8	15.5-23.2

Q1: first quartile; Q3: third quartile.

center for the diagnosis and treatment of thoracic tumors, which might have affected the selection of cases. Twenty-two (76%) of the benign cases were secondary to tuberculosis. This reflects the high prevalence of tuberculosis in Brazil, and the most common presentation of extrapulmonary tuberculosis is the pleural form.[22,23] This high prevalence among the benign pleural exudates was also found in other studies.[20,24,25] In 19 cases (22.3%), it was not possible to differentiate between the benign and malignant cases. Similar results have been described by other authors who reported that, even after cytopathologic study of the pleural fluid and pleural biopsy, approximately 20% of the cases of pleural effusion remain undiagnosed.[26]

The difficulty in making the differential diagnosis between benign and malignant pleural effusion represents a great problem in the study of the condition. Among the potential factors responsible for this difficulty are the inappropriate collection and laboratory manipulation of the pleural fluid, as well as an insufficient quantity of pleural material.[5]

In this context, determination of tumor marker levels represents an auxiliary method of identifying malignant pleural effusion. The potential of using such markers to differentiate between malignant and benign effusions has been mentioned in various studies.[5-9,20,21,27,28] However, it is difficult to draw comparisons among such studies, since there are differences in the number/type of markers evaluated, as well as a significant lack of uniformity in the laboratory methodology and the parameters established as cut-off points to determine specificity/sensitivity, together with the detection of markers in benign diseases and the prevalence of the tumor type in the groups studied. Although some studies have employed a single tumor marker, the combination of more than one marker raises their sensitivity in detecting malignant neoplasms.[5,7,28,29] In the present study, the markers CEA, CYFRA21-1, and CA 15-3 were chosen based on the observation that the majority of the studies have at least two of these in their marker panel, suggesting that their inclusion would increase the diagnostic sensitivity and specificity in the detection of malignant pleural effusion.[7,20,21,29]

In this study, the concomitant determination of serum and pleural levels of the CEA, CYFRA21-1

Table 4 - Cut-off point, sensitivity and specificity of the markers CEA, CYFRA21-1 and CA 15-3.

Biological material/marker	Cut-off point	Sensitivity (%)	Specificity (%)
Pleural fluid			
CYFRA21-1	34.99	69.4	90.0
CEA	1.86	69.4	90.0
CA 15-3	22.38	66.7	90.0
CYFRA21-1 + CEA + CA 15-3	0.333*	80.6	90.0
Serum			
CYFRA21-1	3.12	71.4	93.0
CEA	3.35	57.1	93.0
CA 15-3	30.86	48.6	93.0
CYFRA21-1 + CEA + CA 15-3	0.554*	77.0	93.0

*probability estimated in the logistic regression model.

and CA 15-3 markers had the objective of determining whether the concentrations of these markers were comparable in the two sample types, since the pleural fluid is a blood filtrate and, as such, should be correlated with the blood. However, some authors have reported that the concentrations of tumor markers are greater in the pleural fluid than in the serum, suggesting the local production of some markers, especially those such as CYFRA21-1 (secreted by mesothelial cells), which might alter the overall sensitivity of the marker.[5,11,21]

In the present study, the sensitivity of each tumor marker was established through the construction of ROC curves, opting for specificity equal to or greater than 90%, with the purpose of decreasing the possibility of obtaining false-positive results. Although the ideal specificity is 100% (no false-positives), such an index requires quite high cut-off points, significantly reducing the sensitivity of the markers.[5]

The sensitivity of the three markers in the pleural fluid were similar (CYFRA21-1 = 69.4%, CEA = 69.4%, and CA 15-3 = 66.7%, for a specificity of 90%). When the three markers were used in conjunction in the pleural fluid samples, the sensitivity increased to 80.6%. This finding is in agreement with those of other studies.[5,28-30]

There were differences among the markers in terms of their sensitivity in serum samples (71.4, 57.1, and 48.6% for CYFRA21-1, CEA, and CA 15-3, respectively), with a specificity of 93%. When the three markers were combined, the sensitivity increased, especially for CEA and CA 15-3, justifying the use of this combination (overall sensitivity, 77%).

The levels of CYFRA21-1 and CEA were higher in pleural fluid than in serum ($p < 0.001$ and $p = 0.0014$, respectively). Similar results have been reported in other studies.[5,30] There was no statistically significant difference between the serum and pleural fluid levels of CA 15-3.

For all of the markers studied, there were statistically significant differences between the serum and pleural fluid samples, as well as between the malignant and benign forms of pleural effusion. These results are similar to what has been found in other studies.[5,7,28] In the present study, the use of the tumor marker combination presented significantly greater sensitivity for the identification of malignancy than did the cytopathological study (80.6 *vs.* 69.4%). A similar result was found by some authors.[21] It was not possible to determine the sensitivity of the markers according to the histological type of the neoplasms, since the minimal number of cases did not allow such evaluation.

In the present study, no statistically significant differences were found among the smokers, former smokers and nonsmokers in terms of the tumor marker levels, whether in serum or in pleural fluid. Similar results were reported by other authors who evaluated serum levels of CYFRA21-1.[31] However, some studies have demonstrated that concentrations of CEA are higher in smokers than in nonsmokers, and that high CEA concentrations are associated with the subsequent development of neoplasms.[32]

The ideal marker would be sensitive and specific, as well as reflecting the tumor burden, being predictive of tumor recurrence, and allowing the treatment response to be evaluated.[10] Although the biological markers currently available do not present this profile, they have the advantage of being relatively affordable and are available in the larger cities. The determination of tumor marker levels is not routinely indicated. However, in cases of suspected malignancy and inconclusive initial findings, tumor marker levels should be determined prior to the performance of invasive procedures, thereby optimizing the cost-benefit ratio.

The present study represents an advance in the investigation of pleural effusion at our facility.

Our findings demonstrate that determining serum and pleural fluid levels of the tumor markers CYFRA21-1, CEA, and CA15-3 is useful in differentiating between benign and malignant pleural effusion.

References

1. Light RW. Pleural effusion related to metastatic malignancies. In: Light RW, editor. Pleural Diseases. 4th ed. Philadelphia: Lippincott; 2001.p.108–34.
2. Loddenkemper R. Thoracoscopy-state of the art. Eur Respir J. 1998;11(1):213–21.
3. American Thoracic Society. Management of malignant pleural effusion. Am J Respir Crit Care Med. 2000;162(5):1987–2001.
4. Sahn SA. Pleural diseases related to metastatic malignancies. Eur Respir J. 1997;10(8):1907–13.
5. Romero S, Fernández C, Arriero JM, Espasa A, Candela A, Martín C, et al. CEA, CA 15-3 and Cyfra 21-1 in serum and pleural fluid of patients with pleural effusion. Eur Respir J. 1996;9(1):17–23.
6. Gross JL. Marcadores tumorais séricos em pacientes com carcinoma de pulmão não pequenas células [dissertação].

São Paulo: Faculdade de Medicina, Universidade de São Paulo, 1999.
7. Miédougé M, Rouzaud P, Salama G, Pujazon MC, Vincent C, Mauduyt MA, et al. Evaluation of seven tumour markers in pleural fluid for the diagnosis of malignant effusions. Br J Câncer. 1999;81(6):1059–65.
8. Ferrer JS. Marcadores tumorales em líquido pleural. Arch Bronconeumol. 2000;36(6):295–7.
9. Buccheri G, Ferrigno D. Lung tumor markers of cytokeratin origin: an overview. Lung Cancer. 2001;34 suppl 2:S65–9.
10. Malkin A. Tumor Makers. In: Tannock I, Hill R, editors. The basic science of oncology.2nd ed. Baskerville: McGraw-Hill;1992.p.196–206.
11. Dejsomritrutai W, Senawong S, Promkiamon B. Diagnostic utility of CYFRA 21-1 in malignant pleural effusion. Respirology. 2001;06(3):213–6.
12. Braga ACO. Estudo do antígeno carcinoembrionário no diagnóstico dos derrames pleurais [dissertação]. Rio de Janeiro: Universidade Federal Fluminense, 1990.
13. Alexander JC, Silverman NA,Chretien PB. Effect of age and cigarette smoking on carcinoembryonic antigen levels. JAMA. 1976;235(18):1975-9
14. Light RW. Pleural effusion. N Engl J Med. 2002;346(25):1971-7
15. Light, RW. A new classification of parapneumonic effusions and empyema. Chest. 1995;108 (02):299–301.
16. Agresti A. An introduction to categorical data analysis. New York: John Wiley & Sons; 1996.
17. Hosmer Jr DW, Lemeshow S. Applied logistic regression. New York: John Wiley and Sons; 1989.
18. Fenton KN, Richardson JD. Diagnosis and management of malignant pleural effusions. Am J Surg. 1995;170(1):69–74.
19. Prakash UB, Reiman HM. Comparison of needle biopsy with cytologic analysis for the evaluation of pleural effusion: analysis of 414 cases. Mayo Clin Proc. 1985; 60(3):158-64.
20. Ferrer J, Villarino MA, Encabo G, Felip E, Bermejo B, Vilà S, et al. Diagnostic utility of CYFRA 21-1, carcinoembryonic antigen, CA 125, neuron specific enolase, and squamous cell antigen level determinations in the serum and pleural fluid of patients with pleural effusions. Cancer. 1999;86(08):1488–95.
21. Trape J, Molina R, Sant F. Clinical evaluation of the simultaneous determination of tumor markers in fluid and serum and their ratio in the differential diagnosis of serous effusions. Tumor Biol 2004;25(5-6):276–81.
22. Ministério da Saúde. Fundação Nacional de Saúde. Centro de Referência Professor Hélio Fraga. Sociedade Brasileira de Pneumologia e Tisiologia. Controle de Tuberculose: uma proposta de integração ensino-serviço. 5ª edição. Rio de Janeiro;2002.
23. Seiscento M, Bombarda S. Tuberculose. In: Vargas FS, Teixeira LR, Marchi E, editores. Derrame Pleural. São Paulo: Roca;2004.p.271–84.
24. Valdés L, Alvarez D, Valle JM, Pose A, San Jose E. The etiology of pleural effusions in area with high incidence of tuberculosis. Chest. 1996;109(01):158–62.
25. Ryu JS, Lee HJ, Cho JH, Han HS, Lee HL. The implication of elevated carcinoembryonic antigen level in pleural fluid of patients with non-malignant pleural effusion. Respirology. 2003;08(4):487–91.
26. Ferrer JS, Muñoz XG, Orriols RM, Light RW, Morell FB. Evolution of idiopathic pleural effusion: a prospective, long-term follow-up stu dy. Chest. 1996; 109(6):1508–13.
27. Alatas F, Alatas O, Metintas M, Colak O, Harmanci E, Demir S. Diagnostic value of CEA, CA 15-3, CA 19-9, CYFRA 21-1, NSE and TSA assay in pleural effusions. Lung Cancer. 2001;31(1):9–16.
28. Villena V, López-Encuentra A, Echave-Sustaeta J, Martin-Escribano P, Ortuño-de Solo B, Estenoz-Alfaro J. Diagnostic value of CA 549 in pleural fluid. Comparison with CEA, CA 15.3 and CA 72.4. Lung Cancer. 2003;40(3):289–94.
29. Shitrit D, Zingerman B, Shitrit AB, Shlomi D, Kramer MR. Diagnostic value of CYFRA 21-1, CEA, CA 19-9, CA 15-3, and CA 125 assays in pleural effusions: analysis of 116 cases and review of the literature. The Oncologist. 2005;10(7):501–7.
30. Porcel JM, Vives M, Esquerda A, Salud A, Pérez B, Rodríguez-Panadero F. Use of a panel of tumor markers (carcinoembryonic antigen, câncer antigen 125, carbohydrate antigen 15-3, and cytokeratin 19 fragments) in pleural fluid for the differential diagnosis of benign and malignant effusions. Chest. 2004;126(6):1757–63.
31. Kao C, Hsieh JF, Ho YJ, Tsai SC, Lee JK. Cytokeratin fragment 19 (CYFRA 21-1) in healthy smokers. Anticancer Res. 1999;19(5C):4545–6.
32. Cullen KJ, Stevens DP, Frost MA, Mackay IR. Carcinoembryonic antigen (CEA), smoking and cancer in a longitudinal population study. Aust N Z J Med. 1976;6(4):279–83.